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THE INDUCTION OF PHYSIOLOGICAL TOLERANCE TO PROMAZINE IN MICE

BY

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INTRODUCTION

One of the general principles emerging from the study of physiology is that many of the immediate effects of external agents on the functions of the organism are largely compensated during a prolonged action of the external agent in question. Processes of sensory adaptation, enzymic adaptation, detoxication, antibody formation and various other neural and hormonal regulation mechanisms are involved in these compensatory activities. Phenomena called tachyphylaxis and acquired tolerance are examples of such compensation for the effects of chemical compounds. All these activities can be regarded collectively as instances of *acclimation*, in contrast to the process of true evolutionary adaptation (43).

Tolerance to chemical compounds has been defined in several ways. »Tolerance is a phenomenon characterized by the fact that more and more of a drug must be used to produce equivalent effects» (59). »By tolerance is meant the gradual decrease in the effect produced by repeated administration of a drug; or, conversely, a gradual increase in the dosage of the drug necessary to produce the same effect as did the initial dose» (30). »Acquired tolerance is a phenomenon of cellular adaptation to an alien chemical environment characterized by diminishing biological response» (57).Earlier work on the acclimation to poisons and drugs has been reviewed by Hausmann (27), Tatum and Seevers (59, 60), Heilbrunn (28) and Seevers and Woods (57). In the present study, the acclimative responses associated with the prolonged administration of a drug with prominent metabolic and systemic effects are reported and their probable causes discussed.

Promazine (10-(3'-dimethylaminopropyl)-phenothiazine) is one of the tranquilizing drugs belonging to the neuroleptic or neuroplegic group, to which several other phenothiazine derivatives also belong. Its systemic effects (25, 30, 45, 62, 65) closely resemble those of the better-known tranquillizer, chlorpromazine (Largactil,

Megaphen), studied in detail by Courvoisier *et al.* (12) and subsequently by numerous others.

The intracellular action mechanism of chlorpromazine has been suggested to be the uncoupling of mitochondrial oxidative phosphorylation in the brain (1, 2, 7), probably at the cytochrome c level (6, 14, 15, 58). Subsequently, it has been found that the promazines act as flavin antagonists, thus inhibiting the oxidation of the reduced diphosphopyridine nucleotide (DPNH) (49, 51, 58). In addition, promazines seem to a lesser degree to inhibit the oxidation of ferrocytochrome c (58). The action of promazines as diaphorase flavin antagonists is further corroborated by the observation that promazine inhibits the dinitrophenol-induced ATPase reaction except in low concentrations, which stimulate the reaction (48, 49, 50, 51). Promazines seem to offer a powerful tool for the research on the oxidative phosphorylation (34).

The potassium-stimulated oxygen uptake of the brain cortex slices is strongly inhibited by chlorpromazine (47). The inhibition of the cortical metabolism in the brain is thought to cause an inhibition of the activity of the central nervous system (CNS). On the other hand, a stimulation of the amygdaloid nucleus, which conveys inhibitory impulses to various parts of the brain has been reported (54). A direct inhibition of cellular metabolism, especially of the oxidative phosphorylation in liver mitochondria and of oxygen uptake in liver tissue slices has also been reported (5, 6, 33). A decrease in motor activity, thermoregulatory efficiency, and, subsequently in overall metabolic rate are some of the systemic effects observed (12). The influence of chlorpromazine on the endocrines seems to involve an inhibition of the hypothalamohypophyseal system (10, 35, 52). Chlorpromazine does not seem to have a direct inhibitory effect on the adrenal cortex (42); in prolonged use it may even act as a non-specific stressor (32). The somewhat controversial reports on the effects of neuroplegics on the endocrine system have been reviewed by Laborit (42). The effect of chlorpromazine on the urine secretion is still unclear (36, 39).

The ultimate action of promazines on the nervous cell is far from clear. Chlorpromazine is rapidly taken up by the proteins (perhaps enzyme proteins) in the brain (58). In addition to their properties as diaphorase flavin antagonists and ferrocytochrome c oxidation inhibitors, promazins have been reported to be similar

to sympathicolytics in increasing the potassium concentration inside the cell and thus acting as polarizing agents (41). A detailed and valuable discussion of the properties of promazine neuroplegics is contained in a recent book by Laborit (42).

The literature on the *acclimative responses* evoked by a prolonged administration of promazines is less extensive. Filk *et al.* (22, 23) reported a decrease of the effect of chlorpromazine on the oxygen consumption and body temperature of rats during repeated oral administration. It has been shown that the body temperature control of mice becomes more resistent to the effect of chlorpromazine when this drug is given repeatedly (64). Recently, it was shown that rats develop tolerance to the sedative and lethal effects of chlorpromazine (9).

In order to establish whether mice would develop tolerance against the physiological effects of promazine, the effects of a single dose given to normal animals and a similar dose given to animals which had previously received promazine in smaller doses were compared. The effects on the following variables were recorded: 1) colonic temperature, 2) oxygen consumption, 3) R.Q., 4) motor coordination, 5) motor activity. In addition, the effect of prolonged administration on 6) the lethal dose level was studied. In order to explain the results, measurements of the effect on 7) the oxygen consumption of tissues were performed.

EXPERIMENTAL

1. COLONIC TEMPERATURE AND RESPIRATORY METABOLISM

Experimental Series 1. —Twenty male 3 to 5 months old Swiss albino mice (mean weight 28 g) were divided in two groups of ten. The first group of animals (the control group) received a single subcutaneous injection of 30 mg of promazine hydrochloride (Sparine, manufactured by Leiras, Ltd., with the licence of Wyeth, Inc.) per kg of body weight. 15 minutes before the injection the colonic temperature of the animals was measured with a thermocouple inserted to the depth of 18 mm:s into the colon. The colonic temperatures were also measured at 30, 60, 90, 120, 150, 300, 400, 600, and 800 minutes after the injection. The second group of mice (the experimental group) received 10 mg of promazine hydrochloride per kg daily during a period of 25 days. After this treatment, they received a single dose of 30 mg per kg as did the animals in the control group. The body temperatures were recorded as previously. The results of these experiments are shown in Fig. 1. The experiments were performed at 19°C.

In order to find out, whether the differences between control and experimental animals could be due to the repeated injections as such daily injections of saline were given to five male mice for 22 days. The average effect of a subsequent administration of 30 mg of promazine per kg on the colonic temperature did not significantly differ from the average effect of the same dose given to five other animals, which had never previously received injections. Thus the effect of the repeated administration of promazine was not due to the repetition of the procedure of injection, but to some effect specific to promazine.

On the basis of these data, it was assumed that the prolonged administration of promazine would exert a similar influence on the

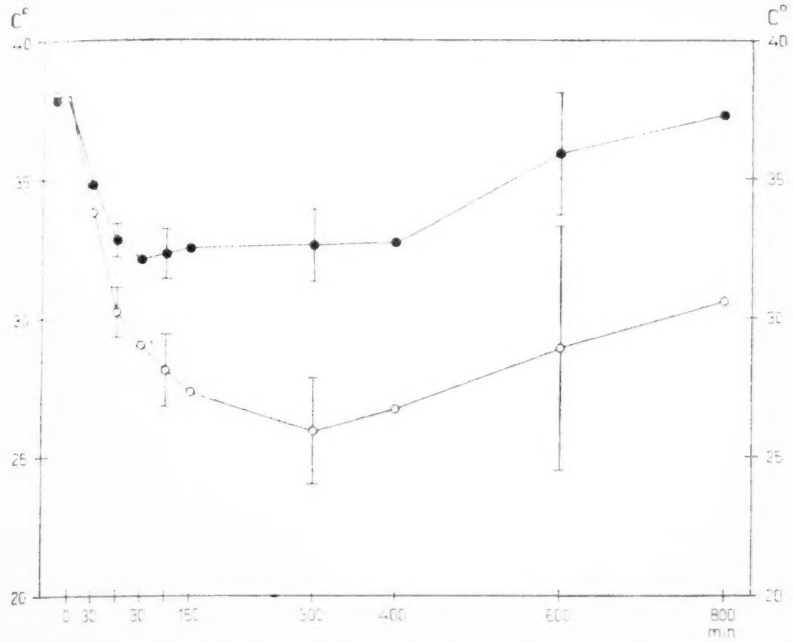

Fig. 1. — The effect of 30 mg per kg promazine on the colonic temperature of mice. Upper curve: the experimental animals. Lower curve: the control group. Verticals at means indicate the standard deviation (± 1 S.D.). Further explanations in the text (Exp. series 1.).

metabolic rate of the animals. This was checked by the Experimental series 2.

Experimental Series 2. — Fifteen male mice were divided into two groups, the first consisting of 8 mice (the control group). The oxygen consumption and R.Q. of these animals were measured by the method of Haldane and Kendeigh, as described previously (45). In order to eliminate the effects of the initial increase in mobility of the animal in the animal chamber, the mice were put in the animal chamber 15 minutes before the actual period of measurement. After two measurements, between which the colonic temperature of the animal was also measured, the animals received subcutaneously 30 mg of promazine hydrochloride per kg. The oxygen consumption, R.Q. and colonic temperature were again measured during two periods of 15 minutes ending 90 and 120 minutes after the injection. The second group of animals (the experimental group) first received 25 daily doses of 10 mg of promazine hydrochloride per kg, and subsequently, the same treat-

TABLE 1

THE EFFECT OF 30 MG PER KG PROMAZINE ON THE OXYGEN CONSUMPTION, R.Q., AND COLONIC TEMPERATURE OF CONTROL AND EXPERIMENTAL MICE. STANDARD ERROR IS SHOWN AT EACH MEAN.

	Oxygen Consumption ml g−1 h−1	R.Q.	Colonic Temperature °C
Control group (8)			
normal values (16)	4.55 ± 0.10	0.82 ± 0.01	38.0 ± 0.1
90 min. after inj. (8) ..	2.20 ± 0.06	0.69 ± 0.01	28.5 ± 1.1
120 min. after inj. (8) ..	1.96 ± 0.05	0.68 ± 0.01	27.2 ± 0.8
Experimental group (7)			
normal values (14)	4.68 ± 0.14	0.79 ± 0.01	37.8 ± 0.1
90 min. after inj. (7)	3.33 ± 0.23	0.71 ± 0.01	32.4 ± 0.3
120 min. after inj. (7)	3.20 ± 0.17	0.70 ± 0.01	32.1 ± 0.3

ment as the animals of the control group. The results are presented in Table 1.

The results of the experimental series 1 and 2 can be summarized thus: The prolonged administration (for 25 days) of promazine decreases the effect of a single dose of promazine 1) on the colonic temperature and 2) on the rate of oxygen consumption. Promazine causes a decrease in the R.Q. The effect of prolonged administration on this decrease seems to be negligible. In all the variables measured, there was no significant difference between the normal values of the control groups and those of the experimental groups. Thus the prolonged promazine administration had not affected the general oxygen consumption, R.Q., or colonic temperature level of the animals.

2. MOTOR COORDINATION AND MOTOR ACTIVITY

Experimental Series 3. — Ten male mice (4 months old, mean weight 29 g) were used as test animals. The capacity for motor coordination was measured with a tilted plane test modified from that earlier designed for the measurement of the level of alcohol intoxication in rats (3). Each animal was placed in a normal position head pointing upwards on a slightly tilted piece of plywood. The other end of plywood plane was lifted cautiously through 90° in 10 seconds. When the animals began to slide down the tilted plane, the angle of the plane to the horizontal was recorded. This was

repeated five times for each animal. From the results the average value for the onset of sliding down was calculated. Fifteen mg per kg promazine hydrochloride was injected subcutaneously into the test animals. The series of five observations was repeated twice for each animal, 15 and 45 minutes after the injection. The animals then received a daily dose of 10 mg of promazine hydrochloride per kg for 22 days. Subsequently, the experiments with the tilted plane were repeated. The results are shown in Table 2. A simple

TABLE 2

THE EFFECT OF 15 MG PER KG PROMAZINE ON THE ABILITY FOR MOTOR CO-ORDINATION AS MEASURED WITH THE TILTED PLANE TEST. FIGURES INDICATE THE AVERAGE ANGLE OF THE PLANE AT THE ONSET OF THE SLIDING DOWN. STANDARD ERROR IS SHOWN AT EACH MEAN.

	Before Injection	15 Minutes after Injection	45 Minutes after Injection
Control	38.9 ± 0.4	37.0 ± 0.2	34.0 ± 0.3
Experimental	41.6 ± 0.3	41.7 ± 0.3	42.1 ± 0.3

variance analysis showed that the differences between the means in the control group were highly significant ($F = 74$, $P < 0.001$), whereas the means for the experimental group did not significantly deviate from eachother.

Experimental Series 4. — Five male mice of the same size and age as in experimental series 3 served as experimental animals and ten others as controls. The motor activity was recorded with the aid of a suspended cage and a kymograph. The normal activity of the animals was recorded for 45 minutes, and, subsequently, the animals received a subcutaneous injection of 10 mg per kg promazine hydrochloride. Their motor activity was again recorded by means of the suspended cage. Before the experiment, the animals of the experimental group received a daily dose of 10 mg per kg for 30 days. All recordings were made between 13.00 and 17.00 hours. Some representative recordings are presented in Fig. 2.

The results of the experimental series 3 and 4 can be summarized thus: The prolonged administration of promazine decreases the effect of a single dose of promazine 1) on the capacity for motor coordination, as measured with the tilted plane test and 2) on the motor activity, as measured by the suspended cage method. On the other hand, the prolonged administration of promazine some-

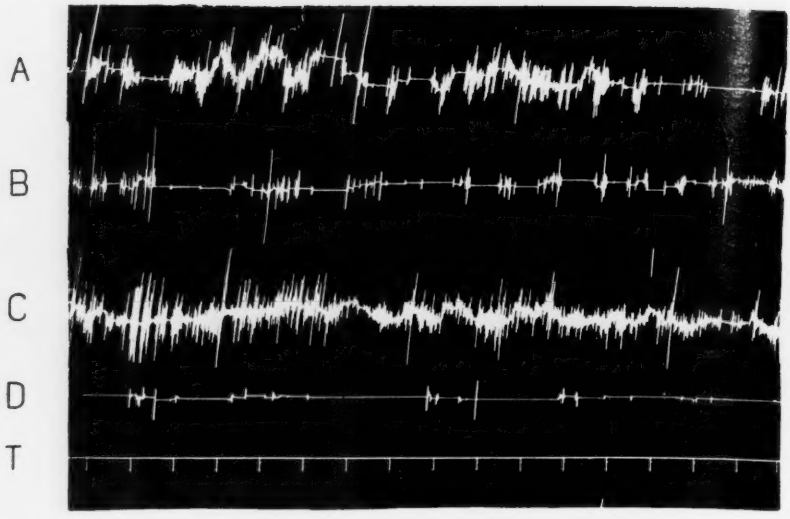

Fig. 2. — The effect of 10 mg per kg promazine on the motor activity. A typical sample of suspended cage records. A — experimental record, normal activity. B — experimental record, activity modified by promazine. C — control record, normal activity. D — control record, activity modified by promazine. T — time in minutes. The sample records begin 15 minutes after the onset of the proper period. Further explanations in the text (Exp. series 4.).

what increases the general level of motor coordination and decreases the normal motor activity level. As slight movements of the animal on the tilted plane often initiated the sliding, the lesser basal mobility of the animals subjected to the prolonged medication may explain the apparently better capacity for motor coordination in these animals. The lesser liability of the experimental animals to the effects of promazine on motor coordination and activity was a further evidence of the tolerance against the physiological effects of promazine developed in these animals in the course of the prolonged administration.

3. LETHAL DOSE LEVEL AND THE BEHAVIOURAL EFFECTS OF SUBLETHAL DOSES

Experimental Series 5. — An estimation of the lethal dose level of promazine in control mice and in those which had received promazine in daily doses of 10 mg per kg for 30 days was made by injecting promazine hydrochloride in doses of 150, 200, 250, and

300 mg per kg into groups of Swiss albino male mice, each group consisting of five mice. For 250 mg per kg, groups of ten mice were used. The mean weight both for control and experimental animals was 32 g and their age 6 months. The pre-treatment of the experimental animals with promazine did not affect their body weight. The experiments were performed at 25°C. The results are shown in Table 3.

TABLE 3.

DATA FOR THE ESTIMATION OF THE LETHAL DOSE LEVEL FOR PROMAZINE.

Dose mg per kg	Number of Dead Animals per Total Number of Animals	
	Control	Experimental
150	1/5	0/5
200	0/5	2/5
250	6/10	2/10
300	5/5	2/5

It appears, that the LD_{50} for promazine seems to be slightly elevated by prolonged administration of this drug.

Some observations made on the behaviour of the animals during the lethal dose level estimation were of interest. The experimental animals were quite active even after the injection of the lethal or sublethal dose. About ten minutes after the injection, the animals had convulsions, which caused them to jump even to a height of 50 cm above the cage bottom. This jumping syndrome also occurred occasionally in the control animals. These rapidly fell in torpor. Some of the control animals were in a torpor even on the next day. These animals usually succumbed. The experimental animals died before the control ones. The surviving experimental mice were very thirsty during the day after the injection. As expected, the colonic temperatures of the control animals fell to lower values than those of the experimental animals. All these observations indicate that the effects on the central nervous system were different in the two groups of animals.

4. OXYGEN CONSUMPTION OF TISSUES

Experimental Series 6. — Thirty Swiss albino male mice (mean weight 30 g, age 5 months) were divided in two groups of fifteen. The animals of the first group served as controls, those of the other received a daily dose of 10 mg promazine hydrochloride per kg for 40 days. The oxygen consumption of the excised diaphragm and liver slices, as well as that of the brain cortex slices was measured by conventional Warburg technique at 37°C, using Krebs-Ringer phosphate saline (pH 7.4) with pyruvate (0,01 M) as the medium. The animals were decapitated, prepared and the tissue slices cut in a cooled room at 0°C. The tissue slices were cut with a simple Deutsch tissue slicer (61, pp. 136—137) to a thickness of about $\frac{1}{3}$ mm. Air was used as the gaseous phase. Some of the vessels prepared for each series contained the above medium with 0.6 mM per liter (or in some cases 1 mM per liter) promazine hydrocloride in it. The results were calculated on the basis of the dry weight of the tissues and expressed as QO_2 (microliters O_2 per mg dry weight per hour) for the first three periods of 30 minutes after the equilibration. The averages of the results are shown in Table 4.

It appears that promazine in 0.6 mM concentration causes

TABLE 4.

THE EFFECT OF PROMAZINE ON THE TISSUE OXYGEN CONSUMPTION. N — EXPERIMENTS WITHOUT PROMAZINE, P — EXPERIMENTS IN THE PRESENCE OF 0.6 MM PROMAZINE *in vitro*. FURTHER EXPLANATIONS IN THE TEXT (EXP. SERIES 6).

	QO_2							
	First 30 Minute Interval		Second 30 Minute Interval		Third 30 Minute Interval		Number of Experiments	
	N	P	N	P	N	P	N	P
Diaphragm								
Control	4.1 ± 0.4	2.5 ± 0.3	3.3 ± 0.4	1.0 ± 0.1	2.5 ± 0.2	0.8 ± 0.1	5	6
Experimental..	4.8 ± 0.5	2.6 ± 0.3	3.8 ± 0.5	1.3 ± 0.3	3.1 ± 0.4	0.8 ± 0.3	5	8
Liver slices								
Control	3.4 ± 0.2	2.7 ± 0.3	3.1 ± 0.3	1.6 ± 0.3	3.0 ± 0.3	1.0 ± 0.2	5	5
Experimental..	3.7 ± 0.2	2.8 ± 0.3	3.1 ± 0.1	2.0 ± 0.2	2.7 ± 0.2	1.3 ± 0.2	6	6
Brain cortex slices								
Control	6.8 ± 0.4	3.9 ± 0.2	7.2 ± 0.4	3.1 ± 0.3	7.1 ± 0.5	2.6 ± 0.3	10	16
Experimental..	8.4 ± 0.5	5.5 ± 0.3	8.5 ± 0.4	5.0 ± 0.5	8.5 ± 0.4	4.2 ± 0.5	10	16

a progressive inhibition of the oxygen uptake in all three tissues studied. A similar progressive inhibition of oxygen consumption by chlorpromazine has been earlier reported for rat and guinea pig brain cortex and for rat heart and liver slices (4, 11, 12, 24, 26, 33, 47, 53). Also promazine has been earlier found to cause inhibition of the oxygen uptake in the rat brain cortex slices (38).

In our experiments the inhibition was most rapid in the diaphragm. The inhibition of oxygen uptake in all the tissues was even more marked when 1 mM promazine hydrochloride was used in the experimental medium. This concentration totally inhibited the respiration within one hour in three out of the seven additional experiments made using this concentration.

TABLE 5

THE PERCENTAGE OF THE INHIBITION OF THE TISSUE OXYGEN CONSUMPTION CAUSED BY 0.6 MM PROMAZINE *in vitro*.

	First 30 Minute Interval	Second 30 Minute Interval	Third 30 Minute Interval
Diaphragm			
Control	39	70	68
Experimental	46	66	74
Liver slices			
Control	21	48	67
Experimental	24	36	52
Brain cortex slices			
Control	43	57	63
Experimental	35	41	51

In Table 5 the mean inhibition of the oxygen consumption by 0.6 mM promazine is expressed as a percentage of the corresponding oxygen uptake measured without promazine in the medium. In the diaphragm there were no significant differences between the inhibition values for control and experimental groups of animals. In spite of the apparently large differences between control and experimental animals in the inhibition caused by 0.6 mM promazine in the respiration of the liver slices, the differences were not statistically significant. The oxygen uptake of the diaphragm, as well as that of the liver slices, was similar, within the limits of error, for the control and experimental animals when measured without the presence of promazine.

More interesting results were obtained in the experiments using brain cortex slices: 1) The oxygen consumption without promazine was significantly higher in the brain cortex slices from animals treated for 40 days *in vivo* with promazine (Table 6). 2) The progressive inhibition of the oxygen uptake caused by promazine *in vitro* was significantly smaller in animals which had previously received promazine repeatedly *in vivo* (Table 7). 3) This difference in the degree of inhibition was apparently about the same as the difference in the oxygen uptake of brain cortex slices, measured with pyruvate as the substrate but without promazine in the medium.

TABLE 6

VARIANCE ANALYSIS OF THE DATA ON THE OXYGEN CONSUMPTION OF THE BRAIN CORTEX SLICES OF CONTROL AND EXPERIMENTAL ANIMALS UNDER NORMAL CONDITIONS.

Source of Variation	DF	Mean Square	F	P
Control vs. experimental ..	1	32.5	5.74	0.05
Between individuals.....	18	5.66		
Between measurements	2	1	3.84	0.05
Remainder	38	0.26		
Total	59			

TABLE 7.

VARIANCE ANALYSIS OF THE DATA ON THE OXYGEN CONSUMPTION OF THE BRAIN CORTEX SLICES OF CONTROL AND EXPERIMENTAL ANIMALS IN THE PRESENCE OF 0.6 MM PER LITER PROMAZINE.

Source of Variation	DF	Mean Square	F	P
Control vs. experimental ..	1	68	21.9	0.001
Between individuals.....	30	3.1		
Between measurements	2	15.5	9.1	0.01
Remainder	62	1.7		
Total	95			

Thus it may be concluded that the oxygen uptake of the mouse brain cortex in the presence of pyruvate is elevated through a prolonged *in vivo* administration of promazine by about 20%. This portion of the respiration is not affected by promazine and thus renders the oxygen uptake of the brain cortex less liable to the progressive inhibition caused by promazine *in vitro*.

DISCUSSION

It appears from the results presented above that the effect of a single dose of promazine on the colonic temperature, respiratory metabolism, motor coordination and motor activity decreases during prolonged administration of promazine in small doses to male mice. It must be pointed out, that these results do not necessarily apply to female mice, because differences in the effects of promazine between the sexes have been found to occur in mice (44) and in man (16).

The differences in the effect of promazine on the control and experimental animals could be the result of compensatory changes occurring during prolonged promazine treatment in one or several of the following systems: 1) the detoxication mechanism for promazine, 2) the endocrine system, 3) the nervous system, 4) the subcellular systems of all reacting tissues. All these systems have been reported to be influenced by phenothiazine derivatives (cf. Introduction).

The repeated administration of chlorpromazine delays the excretion of chlorpromazine in the urine (29). After one subcutaneous injection of 25 mg per kg into the rats, the excretion of chlorpromazine ceased in 84—102 hours. After 8 successive daily injections, chlorpromazine was detected in the urine even 192 hours after the last injection. Thus an enhanced excretion of promazine as such in the experimental animals does not seem to offer a plausible explanation for the acclimation effects found.

Only 1 to 10% of promazines are excreted in an unchanged form (8, 17, 29, 37, 55, 63). For the most part, the promazines are metabolized in the body (18). The major metabolite is promazine sulfoxide (8, 55, 56), which is probably formed in the liver and even further metabolized there. There seem to be no data available on the effect of repeated administration of promazines on their metab-

olism. However, an increase in the rate of promazine metabolism and detoxication would not seem to offer a plausible explanation for the acclimatory changes in the brain metabolism.

The somewhat controversial results of the action of promazines on the endocrine system, as well as the acclimatory responses found, for example, in the motor coordination measurements, make it difficult to explain the acclimation to promazine on the basis of endocrine adjustments. Hence the acclimation of the function of the nervous system seems to offer the most probable alternative as an explanation of the acclimation to promazine. Additional evidence for this conclusion is provided by the tissue respiration measurements. On the basis of the data from those experiments, a tentative explanation may be suggested.

Promazine has been shown to be a powerful inhibitor of the oxidation of DPNH (49, 58). On the other hand, the succinic dehydrogenase pathway to cytochromes is not inhibited by promazine in any system studied (13, 47). If the DPNH oxidation in the cells of the CNS is often subjected to an inhibition, as may occur in repeated *in vivo* administration of promazine, the relative importance of the uninhibited succinic dehydrogenase pathway is likely to increase through kinetic shifts or through an inductive enzymic adaptation. If the succinic dehydrogenase pathway becomes more activated, the oxygen consumption may increase and this portion of the respiration is not affected by promazine, thus rendering to the nerve cells in question a less inhibited oxidation rate even in the case of promazine administration. Thus the apparent effects of promazine will be partly compensated. This seems to occur in the brain cortex, thus allowing a less subnormal energy expenditure for restoration of the membrane potential and for other activities of these cells. The function of the CNS becomes thus less vulnerable by promazine, if this is given in repeated small doses before the actual test; changes in physiological activities showing a corresponding acclimation effect can be explained as the result of this metabolic shift in the cells of the CNS. Thus the changes in motor activity, in motor coordination, and probably also in the lethal dose level may be associated with the change of the metabolism of the CNS cells. The change in the control of the body temperature is probably partly dependent on the activity of the thermoregulatory centre in the CNS, partly on the motor activity

of the animal, and also on the heat production of the organs, *e.g.* the liver, which shows a trend to acclimation of the oxygen uptake similar to that found in brain cortex. The intensity of the respiratory metabolism of the entire animal is again largely dependent on the changes of the body temperature control and thus also on the state of the CNS.

All the reported acclimative responses evoked by prolonged promazine treatment in mice can be explained as secondary effects of the acclimation of the cellular metabolism to promazine, especially in the brain. This may occur by an activation of the succinic dehydrogenase pathway. This hypothesis will be tested by comparisons of the measurements of endogenous oxygen uptake and succinic dehydrogenase activity between control and experimental animals, as well as by measuring the rate of dehydrogenation of other Krebs cycle intermediates in their tissues. It is, of course, also possible that the development of tolerance involves an increase in the activity of DPN-dependent dehydrogenases.

An additional prediction may be made on the basis of the previous data. Barbiturates are known not to inhibit the succinic acid respiration (19, 20, 21). They inhibit the oxidation of DPNH by flavin in mitochondria (21). The effects of barbiturates are potentiated by promazines (40). Thus it seems possible to predict that the acclimation of animals to promazine also renders their physiological functions less liable to the effects of barbiturates. A well-known analogy to this phenomenon of cross-tolerance is the old clinical observation that a subject acclimated to the effects of alcohol also shows tolerance to several general anaesthetics. However, the chronic chlorpromazine administration to rats did not cause a cross-tolerance to pentobarbital (9).

Although several physiological functions of male mice have thus shown to become tolerant to the effect of promazine, it is not certain that a tolerance is developed in man to the therapeutic action of promazine. It can be questioned, for example, whether the motor activity of mice is a valid criterion of the therapeutic action of promazine and not merely comparable to the side-effects of this drug in man.

SUMMARY

1. The effects of a single dose of promazine on several physiological functions of control male Swiss albino mice and of mice which had previously received daily doses of 10 mg promazine hydrochloride per kg body weight for 20 to 40 days have been compared. Altogether 150 animals have been used for the experiments.

2. The prolonged administration of promazine renders the animals less susceptible to the physiological effects of promazine on a) colonic temperature, b) oxygen consumption, c) motor coordination measured with a tilted plane test, d) motor activity as measured with the suspended cage method, e) oxygen consumption of brain cortex slices with pyruvate as the substrate.

3. The prolonged administration slightly elevates the LD_{50} for promazine.

4. The prolonged administration causes a) an increase in the capacity for motor coordination, b) a decrease of motor activity, c) a change in behavioural reactions to high doses, d) an increase in the oxygen consumption of brain cortex slices with pyruvate as the substrate.

5. The prolonged administration causes no apparent change in a) the colonic temperature, b) oxygen consumption, c) R.Q., d) the effect of promazine on R.Q., e) the oxygen consumption of the excised diaphragm, f) the effect of promazine on the oxygen consumption of the excised diaphragm.

6. As the prolonged administration of promazine causes an increase of about 20% in the pyruvate-supported oxygen consumption of brain cortex slices, and as this portion of the respiration is not inhibited by promazine, it is suggested that the acclimation of the physiological functions to promazine is the result of an increase in the activity of the succinic dehydrogenase pathway

in the brain cortex cells. The enhanced activity of this alternative pathway is thus attributed to the prolonged administration of promazine. The increase in the activity of DPN-dependent dehydrogenases would be another possible explanation. The acclimation to promazine is thus assumed to be an intracellular change of the metabolic pattern in the nervous system. The same phenomenon may occur in the liver.

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